IDENTIFICATION OF *ESCHERICHIA COLI* GENES REQUIRED FOR BACTERIAL SURVIVAL AND MORPHOLOGICAL PLASTICITY IN URINARY TRACT INFECTIONS

DANIEL GIUSEPPE MEDIATI

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CERTIFICATE OF AUTHORSHIP AND ORIGINALITY

I, Daniel G. Mediati, certify that the work in this PhD thesis, carried out between March 2014 to July 2018, has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledge within the text.

I also certify that this PhD thesis has been written by me. Any assistance that I have received in my research work and preparation of this thesis has been acknowledged. In addition, I certify that all information sources and literature used are indicated in this thesis.

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Signature of candidate:

Production Note:
Signature removed prior to publication.

Daniel Giuseppe Mediati

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TO MUM AND DAD,

WHO INSTILLED IN ME THE GREAT VALUE OF

EDUCATION.

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The following Supplementary Data Tables can be accessed online with this thesis.

ABBREVIATIONS

A	Absorbance
Amp	Ampicillin
BLAST	Basic local alignment search tool
bp	Basepair
Cm	Chloramphenicol
Δ	Delta (change in)
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTI	Defined trypsin inhibitor
g	Centrifugal force (x unit gravitational field)
GFP	Green fluorescent protein
h	Hours
IBC	Intracellular bacterial community
kb	Kilobase
Km	Kanamycin
L	Liters
LB	Luria Broth
M	Molarity (mol/L)
m	Meters
μ	micro (10^{-6})
Min	The Min protein system
min	Minutes
mL	Mililiters
MOI	Multiplicity of infection
ms	Miliseconds
MW	Molecular weight
n	nano (10^{-9})
NO	Nucleoid occlusion system
OD	Optical density
ORF	Open reading frame
ρ	Density (Rho)
PAI	Pathogenicity island
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
QIR	Quiescent intracellular reservoir
RNA	Ribonucleic acid
RT	Room temperature
rpm	Revolutions per minute

TBE	Tris/borate/EDTA
TraDIS	Transposon-directed insertion-site sequencing
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary tract infections
V	Volts
v	Volume
w	Weight
WT	Wild-type

PUBLICATIONS

This doctoral thesis is a compilation of the work carried out as part of the project "Identification of *Escherichia coli* genes required for bacterial survival and morphological plasticity in urinary tract infections" which resulted in the following written works:

Mediati, D.G., Iosifidis, G. (2016) Lifestyle dynamics of uropathogenic *Escherichia coli* in the urinary tract. *ASM Syntrophy*, 17:3.

Mann, R., Mediati, D.G., Duggin, I.G., Harry, E.J., & Bottomley, A.L. (2017) Metabolic adaptations of uropathogenic *E. coli* in the urinary tract. *Frontiers in Cellular and Infection Microbiology*, 7:241.

Mediati, D.G., Burke, C.M., Ansari, S., Harry, E.J., Duggin, I.G. (2018) High-throughput sequencing of sorted expression libraries reveals inhibitors of bacterial cell division. *BMC Genomics*, 19:781.

Mediati, D.G., Monahan, L.G., Charles, I.G., Duggin, I.G. (2018) Genome-wide comparison of *E. coli* genes required for growth in complex and minimal media. *Journal of Bacteriology*, (manuscript to be submitted).

CONFERENCE PROCEEDINGS

This doctoral thesis is a compilation of the work carried out as part of the project "Identification of *Escherichia coli* genes required for bacterial survival and morphological plasticity in urinary tract infections" which resulted in the following presentations:

East Coast Bacillus Meeting, Wollongong, NSW, Australia 2014
Oral Presentation
Authors: Mediati, D.G., Iosifidis, G., Duggin, I.G.

40th Annual Infection and Immunity, Lorne, VIC, Australia 2015
Poster Presentation
Authors: Mediati, D.G., Duggin, I.G.

13th Annual Bacterial Pathogenesis, Phillip Island, VIC, Australia 2015
Oral Presentation
Authors: Mediati, D.G., Burke, C., Ansari, S., Harry, L., Duggin, I.G.

University of Technology Sydney: 3-minute thesis competition 2015
Oral Presentation
Authors: Mediati, D.G., Duggin, I.G.

East Coast Bacillus Meeting, Wollongong, NSW, Australia 2015
Oral Presentation
Authors: Mediati, D.G., Burke, C., Ansari, S., Harry, L., Duggin, I.G.

Becton-Dickinson (BD) Award Final, Sydney, NSW, Australia 2016
Oral Presentation
Authors: Mediati, D.G., Burke, C., Ansari, S., Harry, E., Duggin, I.G.

East Coast Bacillus Meeting, Sydney, NSW, Australia 2016
Oral Presentation
Authors: Mediati, D.G., Monahan, L.G., Charles, I.G., Duggin, I.G.

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Poster Presentation
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PREFACE

Urinary tract infections (UTIs) are the second most common bacterial infectious disease affecting humans, after pneumonia. A range of pathogens have been implicated in causing UTIs, however strains of uropathogenic *Escherichia coli* (UPEC) are the predominant etiological agents. UPEC originate within the intestine, but have adapted the ability to disseminate and colonise the human urinary tract via a multi-stage intracellular infection cycle within the cells of the bladder. This infection cycle is a complex pathway involving epithelial cell attachment, invasion and intracellular biofilm-like proliferation, leading to the formation of a sub-population of filamentous bacteria. Bacterial filamentation occurs when rod-shaped cells grow without dividing. This filamentation accompanies bacterial dispersal and the rupture of the host bladder cell. UPEC filaments have the potential to revert to bacillary, rod-shaped morphology and can thereafter divide as normal bacterial cells, thereby initiating a new infection cycle. There is also the potential for UPEC to ascend to the kidneys and enter the bloodstream causing urosepsis.

The rapid emergence of antibiotic resistance has greatly influenced the severity of UTIs and financial burden on the health-care sector. This further complicates UTI therapies and highlights the urgent need to advance our understanding of the biological mechanisms and requirements underpinning UPEC survival and pathogenesis.

The work carried out for this PhD thesis aimed to expand our existing knowledge surrounding UPEC survival and morphological plasticity (cell shape changes). Each Chapter within this thesis focused on distinct, but related aspects of this overarching aim. Firstly, we developed and applied a high-throughput sequencing-based method for the genome-wide identification of genes and genomic DNA fragments that induce filamentation in *E. coli*. This revealed genes from several prophages, carbon metabolic pathways, as well as endogenous bacterial genes or loci that have known and novel roles in cell division or bacterial filamentation. A large number of short predicted peptides that trigger filamentation were also identified for the first time, which are not expected to have evolved with the purpose of causing filamentation, but could be used as synthetic, artificial inhibitors of cell division.

A transposon-insertion mutant library in the uropathogenic *E. coli* cystitis isolate UTI89 was constructed at a moderate scale. We combined this library with a modified transposon-directed insertion-site sequencing (TraDIS) technique to define the genes required for UPEC growth and survival in M9-glycerol minimal medium compared to a rich LB medium. We identified 60 mutants with a significant fitness defect and reduced capacity to survive in the M9-glycerol, the majority of which encode gluconeogenic and amino acid catabolism proteins. We also highlight novel differences and several apparent discrepancies in the metabolic requirements between uropathogenic and commensal *E. coli*. Several uncharacterised and UPEC-specific genes were identified that likely underlie metabolic capacities of UPEC strains during infection. Two of these genes, *neuC* and *hisF* were confirmed and verified as important during UPEC infection of human bladder epithelial cells *in vitro*.

Finally, we employed TraDIS to an up-scaled *in vitro* UTI model to identify the UTI89 genes required for survival at distinct phases of the multi-stage bladder infection cycle, focusing particularly on the later infection events, when bacterial physiology appears to most substantially change. This revealed a total of 143, 333 and 322 statistically significant genes required for the IBC, dispersal and recovery phases of bladder cell infection. We additionally characterised the extent and distribution of UPEC filamentation in the dispersal phase of our up-scaled model and implicate, through TraDIS, known and novel cell division regulators to potentially be involved in the UTI-filamentation response pathway. Further, the catalog of genes identified through our TraDIS experiments provides a foundation for further characterisation of UPEC factors needed for survival, both in laboratory culture conditions and during human bladder infection.

The extensive gene functional identification reported in this PhD thesis represents both a substantial resource and some of the first steps towards the understanding of UPEC gene function during infection. This is expected to facilitate the development of treatments for UTIs or other bacterial infections in a future faced with increasing resistance to current antibiotics.